Somatic Serogroups, Capsular Types, and Species of Fecal *Klebsiella* in Patients with Ankylosing Spondylitis

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Received 21 December 1998/Returned for modification 31 March 1999/Accepted 26 May 1999

The purpose of the present study was to find out whether patients with ankylosing spondylitis (AS) carry fecal *Klebsiella* strains that belong to serotypes or species specific for AS. Somatic serotypes (O groups), capsular (K) serotypes, and biochemically identified species were determined for fecal klebsiellae isolated from 187 AS patients and 195 control patients. The controls were patients with fibromyalgia or rheumatoid arthritis. The 638 isolates of *Klebsiella* that were obtained represented 161 strains; 81 from AS patients and 80 from the controls. The average number of *Klebsiella* strains per patient was 1.7 for the AS group and 1.5 for the control group. The most common O group was O1, which was observed for isolates from 23 of 187 AS patients and 24 of 195 control patients. Next in frequency was group O2, which was observed for isolates from 17 AS patients and 15 control patients. Regarding the K serotypes, 59 different types were identified, revealing a heterogeneous representation of *Klebsiella* strains, without a predominance of any serotype. By biochemical identification, *Klebsiella pneumoniae* was the most frequently occurring species, being found in 45 AS patients and 45 control patients. Next in the frequency was *K. oxytoca*, which was observed in 26 AS patients and in 29 control patients. *K. planticola* and *K. terrigena* occurred in only a minority of patients. Altogether, when analyzed either separately or simultaneously according to O groups, K serotypes, and biochemically identified species, no evidence of the existence of AS-specific *Klebsiella* strains was obtained. These findings do not indicate participation of *Klebsiella* in the etiopathogenesis of AS.

The question of whether *Klebsiella* contributes to the etiopathogenesis of ankylosing spondylitis (AS) has remained unresolved. Pros and cons against the role of *Klebsiella* are based on numerous studies of fecal carriage, antibody response, and molecular mimicry, and over the years they have been extensively reviewed and debated (2, 6, 11, 20, 21). One must conclude that, so far, no conclusive, indisputable evidence for participation of *Klebsiella* in the pathogenesis of AS exists. However, an aspect which has not received any attention is the significance of bacterial serotypes in infections due to *Klebsiella* strains. Although several studies of fecal carriage, antibody response, and molecular mimicry, and over the years they have been extensively reviewed and debated (2, 6, 11, 20, 21), there is still no consensus on the role of *Klebsiella* in the etiopathogenesis of AS.

The genus *Klebsiella* can be divided into five species: *K. pneumoniae*, *K. oxytoca*, *K. planticola*, *K. terrigena*, and *K. ornithinolytica*. They all typically express on the cell surface a lipopolysaccharide (LPS; O antigen) and a capsular polysaccharide (K antigen), both of which contribute to pathogenicity. *K. pneumoniae* and *K. oxytoca* frequently cause infections, whereas *K. planticola*, *K. terrigena*, and *K. ornithinolytica* are usually nonpathogenic. The somatic (O) antigens of *Klebsiella* have recently been recognized to divide into nine groups (O1, O2, O2ac, O3, O4, O5, O7, O8, and O12), most of which contain several serotypes (10). O typing of *Klebsiella* strains has rarely been applied to clinical isolates; during the past 40 years only five studies of O typing of *Klebsiella* strains have been published (7, 10, 15, 25, 26). In contrast to the small number of O groups, 77 K serotypes are recognized, and their distributions in clinical samples have been widely studied (5, 9).

The present work was undertaken to clarify the potential significance of serotypes and species of *Klebsiella* in patients with AS. For this purpose, we have analyzed the O groups and K serotypes of different species of fecal klebsiellae isolated from patients with AS and compared the results to those obtained for patients with fibromyalgia (FM) or rheumatoid arthritis (RA).

**MATERIALS AND METHODS**

**Patients.** The study was carried out in two parts. Altogether, 187 patients with AS and 195 control patients were enrolled (Table 1). In part I, 72 patients with AS admitted to the Heinola Rheumatism Foundation Hospital during the period from August to November 1993 were included. The controls were 83 patients with FM enrolled during the same period. In Part II 115 patients with AS were included.
ical identification was carried out as described in 2.

was carried out with the API 20 E system (bioMe´rieux, Marcy-L’Etoile, France).

were subcultured on lactose-containing agar plates. Identification of the strains

LPS.

sis into nontypeable strains with smooth LPS and rough (O 2

O8, and O12) were looked for. Strains that did not react in any of the ELISA

et al. (10). All currently recognized O groups (O1, O2, O2ac, O3, O4, O5, O7,

inhibition enzyme-linked immunosorbent assay (ELISA) as described by Hansen

before centrifugation. All strains with no, weak, or unclear reactions by CCIE

scribed by Monnet and Freney (16).

Klebsiella

Controls (FM)

72 21/51 9.9 42.6 ± 11.2

Controls (RA)

112 47/68 14.1 44.1 ± 10.6

Both parts combined

187 68/119 12.7 43.5 ± 8.3

195 144/51 10.6 51.2 ± 5.4

* Reliable information was not available for all patients.

admitted to Turku University Central Hospital during the period from November 1995 to March 1997 were included. The controls were 112 patients with RA enrolled during the same period. Each AS patient or control was included only once. Patients with FM or RA were chosen as controls since these diseases are similar to AS regarding the need for hospitalization and the use of anti-inflammatory agents, both of which might affect the intestinal flora. The diseases were diagnosed according to the generally accepted criteria. The HLA B27 status was determined for 137 AS patients, with 113 being HLA B27 positive. Patients who were vegetarians or who had received antibiotics during the preceding 2 months were excluded from the study. Also excluded were those with any intestinal disorders (Crohn’s disease, ulcerative colitis etc.), celiac disease, lactose intolerance, or diabetes mellitus.

Isolation of Klebsiella. Stool samples were collected at the time of hospital admission. For part I of the study they were stored for transportation at −20°C and were thawed later, immediately before culture. For part II of the study the samples were cultured within 2 to 6 h after collection. In both parts of the study the initial cultures were done on MacConkey inositol-carbenicillin agar that was less than 72 h old (4). The medium contains inositol as the selective substrate for the growth of klebsiellae and carbenicillin (10 µg/ml) to prevent the growth of other enterobacteria. Within 20 h of incubation at 37°C, klebsiellae appear as red or pink colonies on the agar surface, indicating fermentation of inositol. After incubation, all (≤10 for each patient) differently looking red or pink colonies were subcultured on lactose-containing agar plates. Identification of the strains was carried out with the API 20 E system (bioMérieux, Marcy-L’Etoile, France).

Klebsiella strains were preserved in Protect tubes (STC, Heywood, England) at −70°C until serological and biochemical characterization. The final biochemical identification was carried out as described in Bergey’s Manual of Determinative Bacteriology (12). A utilization test was done only with histamine, as described by Monnet and Frenzy (16).

Serotyping. For each patient with fecal Klebsiella, one strain from those identified as Klebsiella was initially K serotyped. If the following (up to nine) strains from the same patient reacted with the same K antisera, they were considered to be identical. Those that did not react with the same K antisera were typed further by the same strategy.

K serotyping was carried out by countercurrent immunoelctrophoresis (CCIE) by using a modification proposed by Palfreyman (19). All 77 known serotypes (K1 through K72, K74, and K79 through K82) were looked for. An extract described by Oerskov and Oerskov (18) instead of a whole-cell suspension was used as the antigen. The extract was heated only once for 1 h at 100°C before centrifugation. All strains with no, weak, or unclear reactions by CCIE were investigated by the classical quelling reaction. O serotyping was done by an inhibition enzyme-linked immunosorbent assay (ELISA) as described by Hansen et al. (10). All currently recognized O groups (O1, O2, O2ac, O3, O4, O5, O7, O8, O12) were looked for. Strains that did not react in any of the ELISA systems were divided by sodium dodeyl sulfate-polyacrylamide gel electrophoresis into nontypeable strains with smooth LPS and rough (O−) strains without LPS.

RESULTS

In part I of the study fecal Klebsiella strains were isolated from 12 of 72 (17%) AS patients and from 9 of 83 (11%) control patients (Table 2). Among the Klebsiella strains isolated from AS patients, 9 of 18 strains were of group O1; in the controls the corresponding ratio was 3 of 16 (P > 0.05). All other differences, including K serotypes, between strains from AS patients and the controls were even less. Seventeen percent of AS patients and 11% of the controls were carriers of fecal Klebsiella. Due to the low frequency of carriage and to the finding that group O1 strains tended to be more frequent among AS patients, we decided to expand the study to include more AS patients and controls.

In part II, 31% of the AS patients and 41% of the controls harbored Klebsiella in the stool (Table 2). Otherwise, no significant differences between results from the two parts of the study were observed, and the results for both parts combined are presented. Likewise, the results were not affected by the sex of the patients, and the findings from the serological and biochemical identifications are presented together regarding both sexes.

With results from both parts of the study combined, 638 isolates of Klebsiella were obtained: 305 from patients with AS and 333 from those with FM or RA. The isolates turned out to represent 161 Klebsiella strains: 81 from AS patients and 80 from control patients. Forty-eight of 187 (26%) AS patients and 55 of 195 (28%) of the control patients harbored Klebsiella. When analyzed according to sex, the corresponding figures were 20 of 68 (29%) for females with AS and 37 of 144 (26%) for female controls. Among the males, 28 of 118 (24%) with AS and 18 of 51 (35%) of the controls harbored fecal Klebsiella. The average number of Klebsiella strains for the patients with fecal Klebsiella was also quite equal in the two study groups, being 1.7 per patient for patients with AS and 1.5 for the controls (P > 0.05) (Table 2). Altogether, 87 patients had only one strain of fecal Klebsiella, 6 patients had two different strains, 4 patients had three different strains, 3 patients had four different strains, 2 patients had five different strains, and one patient had six different strains.

Regarding disease activity, 57 AS patients had an erythrocyte sedimentation rate of >30. In addition, 23 AS patients had clinically active disease, as determined by a physician’s general clinical evaluation. Among these 80 patients with active disease, 20 (25%) had fecal Klebsiella. The corresponding figure for the 107 other AS patients was 25 (23%), indicating that patients with active disease did not harbor fecal Klebsiella more often than those with inactive AS.

The O-group distribution among the fecal Klebsiella isolates has not previously been reported. Among our strains, O1 is the most frequently occurring O group of fecal klebsiellae; this was followed by O2. These findings are in accordance with studies of clinical Klebsiella isolates from other sources (7, 10, 26).

<table>
<thead>
<tr>
<th>Study part and patient group</th>
<th>No. of patients</th>
<th>No. of Klebsiella isolates identified</th>
<th>No. of Klebsiella strains identified</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total Per patient</td>
</tr>
<tr>
<td>Part I</td>
<td></td>
<td></td>
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<tr>
<td>AS</td>
<td>12/72 (17)</td>
<td>54</td>
<td>18</td>
</tr>
<tr>
<td>Controls (FM)</td>
<td>9/83 (11)</td>
<td>52</td>
<td>16</td>
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<tr>
<td>Part II</td>
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</tr>
<tr>
<td>AS</td>
<td>36/115 (31)</td>
<td>251</td>
<td>63</td>
</tr>
<tr>
<td>Controls (RA)</td>
<td>46/112 (41)</td>
<td>281</td>
<td>64</td>
</tr>
<tr>
<td>Both parts combined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS</td>
<td>48/187 (26)</td>
<td>305</td>
<td>81</td>
</tr>
<tr>
<td>Controls</td>
<td>55/195 (28)</td>
<td>333</td>
<td>80</td>
</tr>
</tbody>
</table>

* Mean; only data for patients with fecal Klebsiella are included.
Altogether, the distribution of O groups (Table 3) does not reveal any difference between strains from AS patients and the controls when the distribution was analyzed either according to the number of strains or according to the number of patients harboring fecal *Klebsiella*. Regarding the K serotypes, 59 different types were identified, revealing a heterogeneous representation of *Klebsiella* strains in the human stool (Table 4). Sixteen of the K serotypes occurred only in AS patients, and 15 occurred only in the controls. In other words, no meaningful difference between the K serotypes of strains from patients with AS and the controls could be observed.

We identified four different *Klebsiella* species. The most common was *K. pneumoniae* (55.9%), followed by *K. oxytoca* (34.2%). These two species are known to be pathogens that cause infections, whereas the other two species observed as a minority of strains, *K. planticola* (8.7%) and *K. terrigena* (1.2%), usually do not cause infections. No difference in the distribution of biochemically identified *Klebsiella* species was observed between strains from AS patients and the controls. Likewise, when analyzed simultaneously according to O groups, K serotypes, and biochemical identification, no evidence for AS-specific *Klebsiella* strains emerged.

The most frequently occurring strain appeared to be *K. pneumoniae* of serotype O2:K31, which was harbored by 11 patients, 6 in the AS group and 5 in the control group, all with RA (Table 5). Only 1 of the 11 strains was from part I of the study, which probably explains why this serotype was not observed in FM patients (which were included only in part I). This serotype comprises 6.8% of all the *Klebsiella* strains identified. Next in frequency was a group of eight strains of *K. oxytoca* of the O1 group and with a nontypeable K antigen (two from AS patients and six from RA patients).

**DISCUSSION**

In the present study no serotype or biochemically identified species of *Klebsiella* specific for AS could be observed. Likewise, the results obtained do not reveal increased levels of excretion or increased rates of carriage of *Klebsiella* in the patients with AS. An important and new observation is the finding that the distribution of fecal *Klebsiella* strains seems to be extremely individual; no clear dominance of any type was observed, and almost each person seemed to have been infected with his or her own specific type of *Klebsiella* strains. This finding includes the fact that 16 K serotypes (22 strains) were observed only in AS patients and not in the controls (Table 4). It remains theoretically feasible that these 16 K serotypes would be AS-specific *Klebsiella* serotypes. However, 15 other K serotypes (18 strains) were observed only in the controls and not in AS patients. Therefore, no substantiated evidence of a claim that those 16 K serotypes would be AS-specific *Klebsiella* serotypes.
specific remains, even though serotype-specific K polysaccharides may be important for Klebsiella-macroage interaction (1). The scattered occurrence of different studies of reactive arthritis following enteric infections caused genesis of AS. This conclusion is based on the findings of would have become apparent in the present study. Klebsiella of patients with AS (6, 20) or healthy subjects (17). We may cultured stool samples were used, the frequency of the study was evident. In part II of the study, in which freshly (6, 20). The possibility that the use of frozen samples in part I of AS patients. However, the true nature of such a dis-

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ACKNOWLEDGMENTS

This study was supported by Academy of Finland and EVO of Turku University Central Hospital.

REFERENCES


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<th>Strain</th>
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<th>According to disease</th>
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<tr>
<td>K. pneumoniae</td>
<td>O2:K31</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>O1(K18)*</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>O1:K36</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>O1:K44</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>O1:K18</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

*These strains are not identical even within the species, since the nontypeable (NT) K antigen could represent several different K antigens.

TABLE 5. Five most common Klebsiella strains observed

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